A PRACTICAL RESEARCH DIGEST FOR TURF MANAGERS

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Rhizosphere Microbiology

The Mysterious World of the Turfgrass Root Zone

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Intensive turfgrass management has traditionally focused on the application of a repertoire of primary and secondary cultural activities to maintain the appearance and functionality of a population of turfgrass shoots to a prescribed standard. In short – we manage the green part! That obsession with the grass shoot reflects what I refer to as "iceberg management." In closely mown turf, roots represent a significant, but largely invisible component of the plant ecosystem that is being managed. Just as the captain as the Titanic discovered the significance of what could not be seen, there is increasing evidence that turfgrass managers must become 'root managers' and that an important element of that management will relate to the plant root-soil microbe interaction.

In an earlier issue of *Turfgrass Trends*, Hull (February 1996) discussed the importance of roots to the grass plant and outlined management strategies for enhancing root growth. Roots function to anchor plants, in the absorption of water and nutrients, as a factor in stress tolerance and as a contributor of organic matter to the soil nutrient pool. Establishing and maintaining healthy

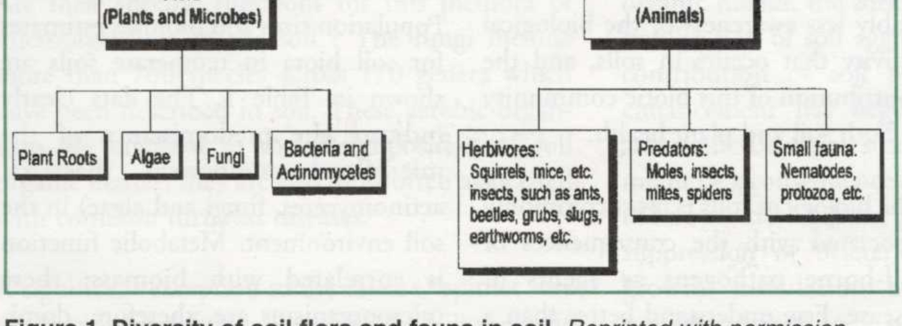

Figure 1. Diversity of soil flora and fauna in soil. *Reprinted with permission from Intro. to Turfgrass Management. UBC Continuing Studies, Vancouver BC.*

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root function is an important part of turfgrass management. That emphasis was reinforced by comments in the September 1997 issue of the Golf Course Superintendents Association of America *Newsline*. Keith Karnok, a University of Georgia turfgrass scientist is quoted, "So much attention is given to the aboveground plant. But the plant that you can see is only as good as its roots. It's imperative to have a good root system, especially during stress conditions such as in a drought system. It's the roots that really save the plant."

The importance of roots may also be a function of their significant contribution to total biomass production in perennial plant species. Studies of natural grassland vegetation confirm that root production is a major component of total biomass. In a managed turf system where regular removal of shoot tissue is the norm, the root contribution to the plant-soil ecosystem might be even more substantial.

The Underground "Black Box"

While we understand much about the relationship between shoot and root growth, and about the response of roots to a variety of management strategies, the interaction of roots with the flora (plants and microbes) and fauna (animals) of the soil remains largely a "**black box.**" There is considerably less awareness of the biological activity that occurs in soils, and the contribution of this biotic community to both soil and plant health.

The biology of soils is most commonly associated with the consequences of soil-borne pathogens as agents of disease. Few understand better than a turf management professional the potentially devastating impact of such diseases on a turf stand. Under intensive management, turf is maintained in a tenuous balance between an attractive playable surface, and disaster. Furthermore, while the consequences of disease are often manifested in deteriorating shoot appearance and performance, many of the causative agents spend all or part of their life cycle in the soil, and use the roots as a vehicle of entry into the plant tissue. However, the prominence of disease as an on-going threat has often diverted our attention away from the beneficial aspects of the turf-soil biological relationship.

The flora and fauna of the soil encompass a diversity of organisms (Figure 1) which participate in essential ecological processes such as organic matter decomposition, nutrient transformations, pathogen antagonism and plant growth promotion. This wide-ranging combination of plant, animal and microbial species form the complex food webs that exist in the soil.

The focus of this article will be to examine the characteristics of the turfgrass root zone, and its interaction with the soil biotic community - particularly the microbial component of that community. That focus on soil microbiology is, in part, a function of the population size in the soil, and the essential metabolic processes associated with these microorganisms. Population sizes and biomass estimates for soil biota in temperate soils are shown in Table 1. The data clearly indicate the predominance of the microfloral component (bacteria, actinomycetes, fungi and algae) in the soil environment. Metabolic function is correlated with biomass; these microorganisms are, therefore, dominant contributors to the metabolic

Table 1. Population and Biomass Estimates for Biota in Temperate Soils

Organisms	*Number/m2*	*Biomass (kg/HFS)*
Bacteria	1013 - 1018	400-5000
Actinomycetes	1012 - 1017	400-5000
Fungi	1010 - 1016	1,000-15,000
Algae	109 - 1014	56-1,000
Protozoa	109 - 1016	17-500
Nematodes	106 - 109	17-400
Earthworms	30 - 7000	100-2,000

HFS = hectare furrow slice. The volume of soil in one hectare approximately 15 cm deep.

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activity that occurs in the soil ecosystem. The data also demonstrate the variability of numerical and mass estimates that have been reported for populations of soil microorganisms. Such variation is, in part, a reflection of the technical challenges of measuring such populations. However, for practical turf management, the significance of variable population size is its relationship to geographic location, soil type and cultural practices. If we are to understand and exploit these microbial populations for the production and maintenance of a healthy turf then it is essential to understand the characteristics of their habitat (the rhizosphere) and the nature of the population (numbers, diversity and metabolic activity).

Microbial Diversity and Competition in Soil

Are their specific functions for this plethora of microorganisms in the soil ? The fungi include more than 700 species across 170 genera which have been described in soil. These aerobic organisms are involved in the decomposition of soil organic matter; they are also most often associated with common turfgrass diseases.

A significant exception to the disease "epithet" are the mycorrhizal fungi - species which invade the plant root and develop a beneficial symbiotic relationship. The plant supplies photosynthetic carbon to the microbe for growth, while the mycelial network of the fungus extends the plant root system into the soil environment. Surface area increases up to ten-fold might result from this mycelial extension. The additional surface area facilitates nutrient uptake of immobile elements such as phosphorus.

Enhanced water and micronutrient uptake, as well as stabilization of soil aggregates, have also been reported as consequences of mycorrhizal activity. Sand profiles are likely to be deficient in mycorrhizal populations However, the development of commercial mycorrhizal inoculants has been limited and responses to inoculation have been variable. The development of mycorrhizal infections appears to occur most effectively through natural inoculation over time.

The aerobic actinomycetes ("filamentous" bacteria) are common inhabitants of moist, warm, well-aerated soils, although they are able to retain activity under drought conditions. Actinomycetes are important contributors to organic matter decomposition, particularly since they can degrade more complex constituents such as cellulose, chitin and phospholipids. In addition, many of these species produce antibiotic compounds `that can suppress or kill associated microbes, and might contribute to some of the natural "biocontrol" relationships observed in soils. There has been little or no attempt to manage the actinomycete component of soils.

The bacterial populations in the soil ecosystem can be significant in number, diversity and metabolic activity. Bacteria play an essential role in nutrient cycling through the breakdown of organic matter, the formation of humus, and the stabilization of soil aggregates. More recently the contribution of soil bacteria to plant growth enhancement has begun to receive additional attention. Bacterial stimulation of plant growth might be a consequence of one or more of a variety of known mechanisms; phosphorus solubilization, suppression of deleterious bacteria, and direct growth stimulation by production of plant growth-promoting substances have been described. The magnitude and importance of these soil bacterial populations have made them the focus of increasing research into their relationship to plant growth and health.

The enormous diversity, both within and among the populations of microbial constituents in the rhizosphere, creates a highly competitive environment, particularly under the substrate limitations that are often a condition of the system. Bacteria tend to thrive on simple organic compounds, typical of the simple exudates released by plant roots into the rhizosphere. Fungal and actinomycete populations are more likely to be enhanced when competition is mediated by the presence of more complex organic compounds.

What is the Rhizosphere ?

The term "rhizosphere' was first introduced by the German scientist Hiltner in 1904 to describe the volume of soil surrounding roots in which bacterial growth is stimulated; we often use the term now to describe "soil under the influence of plant roots." The terminology distinguishes this environment from soil without vegetation, or soil far enough removed from the roots to be outside their sphere of influence. The fibrous root system of a grass stand produces a substantial volume of rhizosphere soil in the turf ecosystem.

While we recognize soil as an important component of the nutrient/water systems that support plant growth, the characteristics of the soil as an environment for microbial life are less well established outside of the scientific community. Soil is a complex habitat for microbial growth with a high ratio of solid (minerals, organic matter and living microbes) to liquid (water). The tripartite solid phase is a complex mixture of components, which exist both separately and in mixed conglomerates. The liquid phase, which conveys nutrients and inhibitors, is normally not continuous in the soil except after rain, snow melt or under extensive irrigation. The discontinuity in this liquid phase can restrict microbial movement and might produce localized accumulations of nutrients and/or toxins, which affect plant growth. Gas movement in the soil might also be restricted by water status, producing localized accumulations of gases such as CO_2 (carbon dioxide) and CH_4 (methane), as well as depleted O_2 (oxygen) levels. These changes can alter the composition and activity of rhizosphere populations by influencing the proportion of aerobes, anaerobes and microaerophiles. Such changes can lead to significantly different kinds of biological activity. For example, the development of black layer under low oxygen tension in areas of the soil.

Microhabitats for Microorganisms

The microbes that inhabit the soil are essentially aquatic organisms - they proliferate in those habitats which have available water. Substantial microbial populations are normally developed in association with the clay and organic matter fractions of the soil system. Clay and other soil separates associate to form aggregates which are stabilized by organic components of the system. These aggregates retain water and develop niches for microbial development. The type of clay minerals involved in this aggregation can influence microbial growth and nutrition, spore germination and competition. It can also provide some physical protection against environmental fluctuations which might expose the organisms to acidity, heavy metals, temperature and desiccation. These microhabitats are highly variable and heterogeneous, but their importance to effective microbial population development is vital.

The development of the sand-based root zone has provided a welcome solution to many problems associated with poor drainage and compaction, but that performance comes at a price. Sand does not retain surface water films effectively, nor does it aggregate easily to develop the microhabitats that are so critical to effective rhizosphere microbial population development. This paucity of microbial activity is particularly critical during the establishment phase of turf. The development of a resilient turf-soil ecosystem with an effective rhizosphere microbial population does not likely occur for three to five years after seeding. The degree of that development is influenced by management and use during that period. The newly seeded, sand-based root zone is normally managed to maintain an adequate (sometimes excessive) supply of plant available nutrients and water. Economic pressures for early use might place additional stress on an otherwise fragile soil ecological environment which is unable to provide the necessary resilience to support the plant population. A significant element of that ecological resilience is contributed by the rhizosphere microbial population. These defective soil ecosystems are analogous to a human with a compromised immune system - they are incapable of an effective response to external challenges (environmental stresses, disease organisms etc.) In this state, many of the strategies which might normally be invoked to treat problems are not only ineffective, but could ultimately exacerbate the imbalances that already exist.

Soil Factors Influence Microbial Populations

In the rhizosphere a variety of factors are known to contribute to the activity, ecology and populations dynamics of soil microbes. Carbon and energy sources, mineral nutrients, growth factors, ionic composition of the soil solution, available water, temperature, atmospheric pressure and composition, pH, surface and spatial relations and the genetics of the microbes themselves all play a role in determining what microbes will proliferate in the rhizosphere.

The rhizosphere environment is characterized by an abundance of mucilaginous material and soluble organic compounds derived from epidermal plant cells and microbial activity. These constituents supply a unique combination of microbial substrates in the rhizosphere. Furthermore, many of these compounds interact with the rhizosphere soil to bind clay and humic aggregates into the secondary structure so essential for the development of microhabitats and enhanced microbial function. This complex heterogeneous environment might be illustrated by looking at the range of activity along the different portions of a root segment (See Figure 2).

Superimposed on this static perspective of the root-soil relationship, is the reality that these rhizosphere relationships are in a constant state of dynamic flux as roots develop and turnover, and as microbial populations evolve. Both spatial and temporal variation in the carbon compounds associated with root development in the soil occur

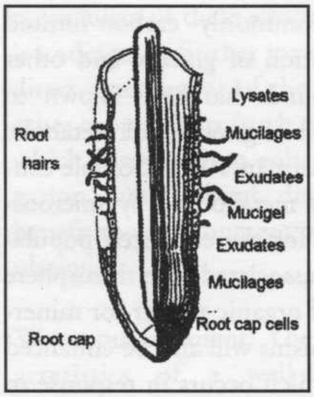

Figure 2. Cross-section of a root showing the general distribution of organic materials in the rhizosphere along the root segment.

Lysates - compounds produced as a consequence of bacterial activity breaking down epidermal cells
Exudates - simple organic compounds released from the plant cells
Mucigel - a complex of high molecular weight mucilages, microbial cells and clay particles
Mucilages - complex organic compounds released by plant cells or from bacterial activity
Root cap cells - released into the surroundings as the root grows; provide organic material for bacterial decomposition.

during normal plant development. Rhizodeposition of root exudates generally decreases with plant age, and increases under soil stresses such as compaction and low nutrient conditions.

The management of most turf systems produces a unique developmental environment in the soil ecosystem. Regular mowing to maintain a vegetative condition produces high levels of root turnover and new root production; as a result, it might be expected that continuing rhizodeposition will occur throughout the growing season.

Microbial Growth in the Rhizosphere

Population size and distribution are influenced by the carbon and energy sources available to microbes, as well as the amounts and availability of nitrogen, phosphorous and sulfur and a variety of naturally occurring growth factors. The mineralization of organic matter is a primary source of the carbon available for microbial growth. Non-specific population growth responses are related to organic matter mineralization. Evidence from the older scientific literature indicates that soil microorganisms are commonly carbon-limited (Newman 1978). Addition of glucose and other simple organic compounds has been shown to result in bursts of microbial growth and metabolic activity (Stotzky and Norman 1961). Soluble exudates which are readily metabolized by microorganisms likely account for the enhanced population sizes that are often associated with rhizosphere soils. The availability of organic matter for mineralization by microorganisms will also be enhanced by the root turnover which occurs in response to regular mowing of turf stands.

Parent (*Golf Course Management*, March 1996) considered the practical implications of carbon limitation in sand-based greens with respect to microbial population sizes. His experience suggests that supplementation with a carbohydrate (sugar)-based fertilizer can enhance the development of rhizosphere microbial populations; the interesting, but as yet scientifically substantiated observation, was that there appeared to be no disproportionate stimulation of possible plant pathogens.

This observation might reflect the ability of bacterial populations to access the sugar substrates more competitively than associated fungal pathogens in the system. The result might be a self-reinforcing feed back system in which enhanced bacterial populations contribute to better soil structure and nutrient turnover, thus improving conditions for both plant growth the development of beneficial microbial habitat.

Water availability is also critical to a healthy rhizosphere microbial population. Where water retention is low, soil microbial (particularly bacterial) activity is correspondingly low. This relationship might be especially critical since fungi can generally metabolize at lower water content than bacteria. Severe drying in the root zone profile might provide a competitive advantage to fungal rhizosphere organisms and increased potential for the development of localized dry spot, as well as enhanced fungal pathogen populations. Once again it is clear that the sand-based profile can create an environment that generates specific challenges for water and rhizosphere management.

Characterizing Microbial Populations

Historically, attempts to define microbial populations have been undertaken using microscopic evaluation of soil preparations, as well as the isolation and culture of microbes on artificial media. While microscopic analysis can provide some valuable evidence for bacterial and fungal biomass estimates, the procedures are not easily adapted for practical management. Culture techniques typically only sample a small fraction of the total population (<10%) which might not even be representative of the active microbial components. Functional activity in the rhizosphere is not necessarily linked to our ability to isolate and characterize individual species.

In the last decade considerable progress has been made in addressing the functional nature of soil microbial populations. Garland and Mills have used redox technology to assess community-based carbon source utilization characteristics of microbial populations. Analysis of the metabolism of 95 different carbon substrates has been used to provide a pattern of metabolic activity that appears to be characteristic of a particular microbial community. This Biolog™ technology couples microbial respiration with an easily measured and quantified dye color change. We have been working to develop an adaptation of the Biolog™ system to investigate microbial carbon use patterns in golf greens and other turf systems. Our preliminary results suggest that microbial populations vary with the season (not surprisingly), and in response to stress and management.

We are currently using this measurement technique to evaluate the establishment and early growth of 'Penncross' bentgrass on various amended sands receiving nitrogen supplementation from an inorganic vs. an organic source, and in the presence and absence of a carbohydrate supplement to stimulate the microbial populations. The strength of this measurement approach is that it is relatively rapid (3 days), does not depend on the ability to isolate and culture the microbes, and reflects the functional activity of the population rather than its growth characteristics. If the technique proves to be a reliable tool for rhizosphere evaluation, and particularly, if it can be used to predict rhizosphere health, it could become an essential feature of rhizosphere microbial management.

Managing the Microbes Can It Be Done ?

The biological and ecological fine points of rhizosphere plant-microbe interactions are clearly an interesting field for scientific study. However, are we any nearer transferring that knowledge and/or technology into the hands of the end user for active management ? I believe that we are.

I have repeatedly emphasized that amended sand greens begin life as a microbial wasteland, and that they do not provide a congenial habitat for healthy rhizosphere microbial growth and development. Management strategies should, therefore, be targeted at altering that imbalance to improve the rhizosphere environment as early as possible in the establishment of the turf.

Design changes: If the amended sand green is our currently accepted technology, what can we do to improve the rhizosphere habitat ? There are currently a number of inorganic amendments (e.g. zeolites, diatomaceous earth, calcined clays, pumice etc.) being used and tested in combination with sand to improve aeration porosity, as well as water and nutrient holding capacity in the sand. In addition to changes in the water and nutrient relationships, the exchange capacity binding sites and the pore spaces in materials such as zeolites might provide habitat potential for microbial development that is unavailable in the current amended sand mixes. Furthermore, in observations of demonstration trials on several golf greens in British Columbia, there is anecdotal evidence that zeolite-amended sand might confer some disease tolerance to the turf. Such observation is consistent with concept that the improved microbial habitat provided by the pore spaces creates enhanced microbial populations that contribute to a disease suppressive effect. We have also observed a direct inhibitory effect of zeolites on the growth of some fungal pathogens in laboratory tests. The mechanism of these effects remains unknown, but is undergoing further investigation. In addition to direct amendment of the sand profile, the use of other supplements (such as humic acid derivatives) which contribute to enhanced soil particle aggregation, and habitat development, might also benefit the development of healthy microbial populations.

Water management: The effective drainage characteristics of a well-designed sand profile encourage the regular use of water, particularly during the critical establishment and grow-in phases. As a result, sand-based turf often receives an abundance of water at intervals that are less conducive to the development of an extensive root system, or a stable rhizosphere microbial population. Shallow root growth is more likely to be associated with decreased stress tolerance and a rhizosphere microbial population which is smaller, less diverse and less resilient to environmental fluctuations. As Richard Hull noted in his earlier article (*Turfgrass Trends* February 1996) the plant response to soil drying is to divert photosynthetic resources to enhance root growth. Water management which can provide for mild drought stress between irrigations produces more deeply rooted turf with a greater potential for stress tolerance and a healthier rhizosphere microbiology.

Fertility management: The water and fertility management demanded to maintain a newly established amended sand turf is not conducive to rapid establishment of a stable root-microbe ecosystem. The use of amendments which can enhance nutrient and water holding capacity and contribute to improved microbial habitat will help to establish a vigorous efficient rhizosphere as soon as possible. Since there is no scientific basis to differentiate the nutrient "quality" of synthetic and organic fertilizers, the contribution of the latter class of product might be a function of the "non-nutrient" constituents and their influence on microbial populations. An extension of the idea that fertilization should include a strategy to address the rhizosphere microbial population has resulted in the generation of carbohydrate-based products specifically targeted at enhancing microbial growth. To date there is limited scientific evidence confirming the efficacy of such products. However, the philosophy underlying their development is consistent with an ecological perspective for effective turfgrass management - that improved microbial activity in the rhizosphere will enhance nutrient cycling, organic matter breakdown and improve soil structure. The enhanced soil ecosystem which is developed will support a healthier, more resilient turfgrass stand.

The turf management world is changing. Increased regulation, increased emphasis on "natural" turf management, and the continuing pressure to maintain turf quality make the professional manager's task a challenging one. Broadening the scope of our management attention to include the mysteries of rhizosphere microbiology will not ease the task, but it will become an increasingly significant component of the management of sustainable turfgrass ecosystems.

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Glossary

Actinomycetes - Soil microorganisms intermediate between the bacteria and true fungi that normally produce a branched mycelium (hence the term "filamentous" bacteria).

Aerobic - Organisms or reactions that require the presence of oxygen in the atmosphere.

Anaerobic - Organisms or reactions that occur in the absence of oxygen.

Microaerophilic - Organisms or reactions that are adapted to low oxygen conditions (but do not function in aerobic or anaerobic environments).

Mycelium - Stringlike filaments of cells characteristic of the growth pattern of the true fungi and actinomycetes. These filaments (hyphae) might be branched or unbranched.

Redox technology - Redox reactions refer collectively to metabolic processes that involve reduction and oxidation (transfer of electrons) - common elements of respiration. These reactions are easily linked to compounds which change color when oxidized or reduced and are convenient ways to measure respiration as a reflection of metabolic activity in environmental samples.

Rhizodeposition - The release and deposit of a variety of carbon compounds and root cell residues along the surface of the developing root, and into the adjacent soil environment.